

09/813444

cont. of 09/847663 Aband

after 1st action

WHAT IS CLAIMED IS:

Elected 1-37

5844344

5348867

Same claims as
09/847663
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Diff. inventor

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1. A method for selecting a polypeptide from a plurality of candidate proteins, the method comprising the steps of:

- (a) obtaining a library of vectors that encode a plurality of distinct candidate polypeptides, wherein said vector provides for the cell surface expression of said candidate polypeptides;
- (b) expressing each of said plurality of candidate polypeptides on the surface of a host cell; and
- (c) selecting a host cell that expresses a desired polypeptide.

E. coli

antibody

1-6

11, 13-16

2. The method of claim 1, wherein said host cell is a Gram negative bacterium.

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3. The method of claim 2, wherein said host cell is *E. coli*.

4. The method of claim 1, wherein said polypeptide is selected from the group consisting of an antibody or antibody fragment, an enzyme, a cytokine, a transcription factor, a clotting factor, a chelating agent, a hormone and a receptor.

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5. The method of claim 4, wherein said polypeptide is an antibody or antibody fragment.

6. The method of claim 5, wherein selecting a host cell that expresses a desired antibody comprises the steps of:

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- (a) contacting said antibody- or antibody fragment-expressing cells with a selected antigen; and
- (b) identifying a host cell that binds to said selected antigen.

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7. The method of claim 6, wherein the antigen is labeled.

8. The method of claim 7, wherein the label is a fluorescent or chemilluminiscent label.

9. The method of claim 6, wherein said selected antigen is located on the surface of a cell other than said host cell, and said host cell that binds to said selected antigen is identified by a method comprising the steps of:

- (a) contacting said host cell with said cell expressing or having conjugated thereto said selected antigen; and
- (b) identifying a host cell bound to said cell expressing or having conjugate thereto said selected antigen.

10. The method of claim 9, further comprising size sorting of bound cells following the step of contacting said host cell with said cell expressing or having conjugated thereto said selected antigen.

11. The method of claim 6, wherein said vector library is obtained by a method comprising the steps of:

- (a) administering to an animal an immunologically effective amount of a composition comprising a selected antigen;
- (b) obtaining from the animal a plurality of distinct DNA segments that encode distinct antibodies or antibody fragments; and
- (c) incorporating said plurality of DNA segments into a plurality of expression vectors, the vectors expressing antibodies or antibody fragments on the outer membrane surface of a Gram negative host cell.

12. The method of claim 11, wherein said plurality of DNA segments are obtained by a method comprising the steps of:

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- (a) ✓ isolating mRNA from antibody-producing cells of said animal;
 - (b) ✓ amplifying a plurality of distinct RNA segments using a set of nucleic acid primers having sequences complementary to antibody constant region or antibody framework region nucleic acid sequences; and
 - (c) ✓ preparing a plurality of distinct DNA segments having sequences complementary to said amplified RNA segments.

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✓ 13. The method of claim 1, wherein said vector library is obtained by a method comprising the steps of:

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- (a) ✓ obtaining a DNA segment that encodes a selected polypeptide;
 - (b) ✗ mutagenizing said DNA segment to provide a plurality of DNA segments that encode a plurality of polypeptides; and
 - (c) — incorporating said plurality of DNA segments into a plurality of expression vectors, the vectors expressing a plurality of polypeptides on the surface of a Gram-negative host cell.
- Mentioned earlier*

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✓ 14. The method of claim 13, wherein said polypeptide is an antibody or an antibody fragment.

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✗ 15. The method of claim 14, wherein said selected cells that express a desired antibody are subjected to cleavage to release the selected antibody or antibody fragment from the surface of the outer membrane.

cleavage

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16. The method of claim 13, wherein selecting a host cell that expresses a desired antibody comprises the steps of:

- (a) contacting said antibody- or antibody fragment-expressing cells with a selected antigen; and
- (b) identifying a host cell that binds to said selected antigen.

17. The method of claim 16, wherein said selected antigen is linked to a detectable label.

18. The method of claim 17, wherein said selected antigen is linked to a fluorescent label, a chemilluminiscent label, a radioactive label, biotin, avidin, a magnetic bead or an enzyme that generates a colored product upon contact with a chromogenic substrate.

19. The method of claim 18, wherein said cells that bind to said selected antigen are identified by a method comprising the steps of:

- (a) contacting said plurality of cells with said detectably labeled antigen under conditions effective to allow specific antigen-antibody binding;
- (b) removing non-specifically-bound antigen from said cells; and
- (c) identifying the antibody- or antibody fragment-expressing cells by detecting the presence of the bound detectable label.

20. The method of claim 19, wherein said cells that bind to said selected antigen are identified by a method comprising the steps of:

- (c) contacting said plurality of cells with a fluorescently labeled antigen under conditions effective to allow specific antigen-antibody binding;
- (d) subjecting said cells to automated cell sorting; and
- (e) identifying the desired antibody or antibody fragment by detecting the fluorescently labeled sorted cells.

21. The method of claim 20, wherein said cells are subjected to sorting by flow cytometry.

22. The method of claim 20, wherein said cells are subjected to a first and a second round of automated cell sorting.

23. The method of claim 22, wherein regrowth of sorted cells is conducted between said first and said second rounds of cell sorting.

24. The method of claim 22, wherein said cells are subjected to a third and a fourth round of automated cell sorting.

25. The method of claim 18, wherein said selected antigen is linked to a magnetic bead.

26. The method of claim 25, wherein cells that band said antigen are selected are identified by a method comprising the steps of:

- (a) contacting said plurality of cells with said magnetic bead labeled antigen under conditions effective to allow specific antigen-antibody binding;
- (b) subjecting said cells to magnetic sorting; and
- (c) identifying the desired antibody- or antibody fragment by detecting the magnetic bead labeled sorted cells.

27. The method of claim 4, wherein said polypeptide is an enzyme.

28. The method of claim 27, wherein said cells expressing a desired enzyme are selected on the basis of enzyme activity.

29. The method of claim 28, wherein said enzyme activity is substrate cleavage.

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These claims were not disclosed in the original parent applications

30. The method of claim 29, wherein cleavage of said substrate results in loss of quenching of a detectable signal.
- 5 31. The method of claim 28, wherein said enzyme activity is substrate binding.
32. The method of claim 31, wherein said binding results in the quenching of a detectable signal.
- 10 33. The method of claim 31, wherein said binding results in the generation of a unique signal not found in the absence of binding.
34. The method of claim 28, wherein said enzyme activity results in the association of a detectable signal with said host cell.
- 15 35. The method of claim 34, further comprising sorting said host cell by flow cytometry.
36. The method of claim 35, wherein said cells are subjected to a second round of automated cell sorting.
- 20 37. The method of claim 36, wherein regrowth of sorted cells is conducted between said first and said second rounds of cell sorting.
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- 25 38. A method for catalyzing a chemical reaction, comprising the steps of:
- (d) obtaining a host cell that expresses an enzyme or catalytic antibody on the surface of the outer membrane; and
 - (e) contacting said host cell with a sample containing the necessary substrates for said chemical reaction.
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39. The method of claim 38, wherein said host cell is a Gram negative host cell.
40. A method for stimulating an immune response, comprising administering to an
5 animal a pharmaceutical composition comprising an immunologically effective amount of
a host cell that expresses an antibody or antigen-combining antibody fragment on the
surface of the outer membrane.
41. The method of claim 40, further comprising the step of obtaining from said animal
10 an antibody.
42. The method of claim 41, wherein said host cell is a Gram negative host cell.
43. An isolated and purified antibody, or fragment thereof, that binds immunologically
15 to digoxin, but does not bind immunologically to digitoxin.
44. A single-chain antibody that binds immunologically to digoxin, but does not bind
immunologically to digitoxin
45. A host cell that expresses, on its cell surface, a single-chain antibody that binds
20 immunologically to a digoxin, but does not bind immunologically to digitoxin.